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The role of substance P and bradykinin in the cough reflex and bronchoconstriction in guinea-pigs

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Abstract

In this study we investigated the ability of aerosolized substance P to induce either cough or bronchoconstriction in guinea-pigs. We have also examined whether pre-treatment, by the inhaled route, of animals with a combination of the neutral endopeptidase inhibitor, phosphoramidon (10^{-3} M), and the diaminopeptidase IV inhibitor, diprotin A (10^{-3} M), enhances the airway response to substance P. Moreover, we also assessed whether aerosol pre-treatment of guinea-pigs with either substance P or bradykinin, at 10^{-4} M, affects the citric acid-induced cough and/or bronchoconstriction. Challenge of guinea-pigs with substance P only at 10^{-3} M resulted in significant bronchconstriction but only a weak and variable cough response (1.1 ± 0.6; $P>0.05$). Pre-treatment of guinea-pigs with both phosphoramidon and diprotin A resulted in a small non-significant increase in the cough response (2.8 ± 0.9 vs. 1.1 ± 0.6; $P>0.05$) but significantly enhanced substance P-induced bronchoconstriction ($P<0.05$). Moreover, exposure of guinea-pigs to substance P (10^{-4} M) prior to citric acid challenge (0.6M) resulted in a significant ($P<0.05$) enhancement of the citric acid-induced bronchoconstriction but not the citric acid-induced cough (11.7 ± 1.8 vs. 12.8 ± 1.5; $P>0.05$). In contrast, exposure of guinea-pigs to bradykinin (10^{-4} M) prior to the citric acid challenge resulted in a significant enhancement of the cough response (9.2 ± 1.9 vs. 25.8 ± 2.5; $P<0.05$) but not the bronchoconstriction ($P>0.05$). These data do not support a major peripheral role for substance P in the cough reflex, although bradykinin is able to sensitize the cough reflex. Furthermore, these data suggest that bronchoconstriction, induced by citric acid, is not responsible for the cough associated with this irritant.

Keywords: Substance P; Bradykinin; Citric acid and cough

1. Introduction

Cough is generally considered as reflex defensive mechanism which is initiated through activation of sensory nerves in the wall of the respiratory tract, from the larynx down to the bronchi, in response to mechanical, chemical or inflammatory mediator stimulation (Adcock, 2003). Despite cough being a very common problem, the mechanisms contributing to this symptom remain poorly understood. However, there is evidence to support the involvement of airway vagal afferents such as sensory C-fibres and rapidly adapting receptors in the cough reflex, as well as other symptoms of respiratory diseases such as bronchospasm (Adcock, 2003).

In addition to their afferent function, C-fibres have also been shown to have an efferent function. These non-myelinated C-fibres contain the tachykinins substance P, neurokinin A and neurokinin B which, upon release, act on NK_1, NK_2, NK_3 receptors respectively to mediate several functions (Regoli et al., 1994). Whilst inhalation of citric acid stimulates both C-fibres and rapidly adapting receptors (RARs), capsaicin appears to stimulate only C-fibres and both these agents have been shown to induce cough, in several species including man, and also bronchoconstriction (Lalloo et al., 1995; Yasumitsu et al., 1996; Kondo et al., 1998; Undem et al., 2002; El-Hashim et al., 2004). Additionally, substance P has also been reported to stimulate RARs

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(Prabhakar et al., 1987; Matsumoto et al., 1994; Bonham et al., 1996) which are also reported to be involved in cough (Canning, 2002).

The fact that neurokinins have been reported to induce cough and that citric acid and capsaicin cause airway neuropeptide release, induce cough and bronchonstriction (the latter two being blocked by neurokinin receptor antagonists) has led to the belief that the peripherally released neuropeptides may be directly responsible for mediating both cough and bronchoconstriction induced by these irritants and that the bronchoconstriction may also, indirectly, enhance the cough response to these irritants (Kohrogi et al., 1988; Takahama et al., 1993; Ujiie et al., 1993; Yasumitsu et al., 1996).

A role for substance P in the peripheral initiation of the cough reflex is also suggested by several other lines of evidence. Tussive stimuli induce substance P release (Martins et al., 1991a,b) and exposure of guinea-pigs to substance P, in concentrations as low as 10^{-17} M, has been reported to induce a cough response (Kohrogi et al., 1988; Takahama et al., 1993, 1995). Furthermore, the neutral endopeptidase inhibitor, phosphoramidon, has been reported to enhance both substance P and histamine-induced cough (Sekizawa et al., 1995; Takahama et al., 1995). Finally, it has been shown that the neurokinin NK_1 receptor antagonist and the dual NK_1 and NK_2 peptide receptor antagonists, N^2-[(4R)-4-hydroxy-1-(1-methyl-1 H-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide) (FK888) and (*N*-[N^2-[2,3-didehydro-*N*-methyl-*N*-[*N*-[3-(2-penthylphenyl)-propionyl]-L-threonyl]tyrosyl]-L-leucynyl]-D-phenylalanyl]-L-allo-threonyl]-L-asparaginyl]-L-serine-v-lactone) (FK224) respectively, are both effective anti-tussive agents in different types of guinea-pig models of cough (Ujiie et al., 1993; Sekizawa et al., 1995; Xiang et al., 1998), when administered by the inhaled route. Taken together, these studies would suggest a role for substance P in cough and would also imply that the mechanism of action of this peptide may be through a local airways effect.

Not all studies however, have been able to confirm that exposure to substance P, by the inhaled route, induces a cough response. For example, exposure of guinea-pigs to substance P at 10^{-4} M (Fox et al., 1996a,b) or pigs, at 10^{-5} M, (Moreaux et al., 2000) does not result in a cough response. Similarly, no cough response has been reported when normal humans inhale substance P (Joos et al., 1987). Thus the peripheral role of substance P in eliciting the cough reflex is far from clear.

The release of some inflammatory mediators in the airways, such as substance P and bradykinin, may not only induce cough or bronchoconstriction but may also sensitize these airway responses to other stimuli (Fox et al., 1996a,b; Moreaux et al., 2000; Boichot et al., 1996). However, whether exposure of animals to inflammatory mediators results in the simultaneous enhancement of both cough and bronchoconstriction is not known.

The aims of this study were to investigate the ability of substance P to induce cough and/or bronchonconstriction in guinea-pigs and whether inhibition of the enzymes neutral endopeptidase and diaminopeptidase IV, by phosphoramidon and diprotin A respectively, can enhance the effect of substance P-induced cough and/or bronchconstriction. Futhermore, we also investigated whether bradykinin and substance P can cause simultaneous enhancement of citric acid-induced cough or bronchoconstriction.

2. Methods

The methods described in this study were approved by the Animal Welfare Committee and Use of Laboratory Animals in the Health Science Centre, Kuwait University.

Conscious, unrestrained, male Dunkin Hartley guinea-pigs, weighing 300–500 g, were placed individually in a transparent plastic whole body plethysmograph (Buxco, Troy, NY) and exposed to nebulized aqueous solutions containing various agents. Aerosols were produced by a DeVilbiss aerogen ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA) and had an aerodynamic mass median diameter range of 1–5 μm (manufacturer's indication). About 0.3 ml of the solution was nebulized per minute.

A pneumotachograph, with defined resistance in the wall of the main chamber, acted as a low-pass filter and allowed thermal compensation. The chamber was also fitted with a microphone and connected to both an external speaker and a computer to allow visualization of the sound signal. The plethysmograph was also connected to a bias flow generator that was supplying air at a rate of 3 l/min and withdrawing air at a rate of 4 l/min. The difference being taken up by airflow into the box through the pneumotachograph. Assessments of cough and airway obstruction were performed simultaneously in the same animal.

2.1. Recording of cough

The animals were continuously watched by a trained observer who counted the number of coughs. The criteria for cough were the characteristic high sound with the mouth open and by a particular pattern in the sound signal. Moreover, during cough there were quick and large abdominal movements, and these were detected as very large and transient increases in airflow over and above the normal flow.

2.2. Measurement of airway obstruction

Airway function was measured by a recently described and validated method (Hamelmann et al., 1997; Chong et al., 1998). It essentially involved use of barometric whole

body plethysmography (WBP) to measure airway caliber. The reasons for choosing this methodology are as follows: (1) it allows measurement of both cough and airway bronchoconstriction simultaneously, (2) the animals are not anesthetized and hence the complications of anesthesia are eliminated, (3) the drugs are administered via the inhaled route which simulates natural exposure to airborne particles, (4) there is now good evidence that this methodology is a good tracker of airway caliber and that Penh correlates well with other indices of airway function such as airway resistance (Hamelmann et al., 1997; Chong et al., 1998; Trifilieff et al., 2000a,b; El-Hashim et al., 2004).

In short, the pressure differences between the main chamber of the body plethysmograph containing the animal and a reference chamber (box pressure signal) were measured. The resulting box pressure signal is caused by volume and resultant pressure changes in the main chamber during the respiratory cycle of the animal. From these box pressure signals, the phases of the respiratory cycle, peak inspiratory pressure (PIP), peak expiratory pressure (PEP), tidal volumes and an index of airway caliber, enhanced pause (Penh), can be calculated:

$$\text{Penh} = \text{Pause} \times \frac{\text{PEP}}{\text{PIP}}$$

Penh is a dimensionless value that reflects changes in the waveform of the box pressure signal from both inspiration and expiration and combines it with the timing comparison of early and late expiration (Pause).

2.2.1. Calibration

The system was calibrated by taking a 2 point reading (high and low) for every signal coming to the software. The calibration system uses a −5 V to +5 V range. This range ensures that every possible variation coming from our signal will be read by our computer and uses the available voltage to the fullest. The calibration is performed with the low signal being 0 ml and a rapid injection of 5 ml of air, via a syringe, into the main chamber of the WBP for the high signal.

2.3. Drugs

Citric acid was obtained from BDH laboratory (Poole U.K.) whilst substance P (acetate salt), phosphoramidon, diprotin A, phosphate-buffered saline, bradykinin were obtained from Sigma (Dorset, U.K.). Citric acid and substance P were made up in 0.9% saline. Phosphoramidon was dissolved in 6.6% ethanol. Diprotin A and bradykinin were both dissolved in distilled water.

2.4. Experimental protocol

All animals were randomly selected. Prior to any aerosol challenges, all animals were allowed a settling period in the body plethysmograph, after which baseline airway function was recorded for 2 min before aerosol challenges commenced. In protocol 2.4.1 and 2.4.2 cough was recorded during the 10 min exposure, and also 5 min post-exposure, to either substance P or citric acid (total of 15 min cough recording). However, in protocol 2.4.3, in addition to recording the cough numbers during the 10 min exposure to citric acid (0.6 M) and 5 min thereafter (total of 15 min cough recording), we have also reported the cough counts during the 10 min pre-treatment with either substance P (10^{-4} M), bradykinin (10^{-4} M) or their vehicles.

2.4.1. Challenge of guinea-pigs with substance P alone

4 groups were established. Three of these groups were exposed to substance P, alone, at 10^{-10} M (group A; n=6), 10^{-4} M (Group B; n=5), 10^{-3} M (Group C; n=11) for 10 min. A fourth group received the vehicle for substance P (0.9% saline). Guinea-pigs were monitored for 5 min thereafter.

2.4.2. Challenge of guinea-pigs with substance P following pre-treatment with phosphoramidon and diprotin A and challenge of guinea-pigs with citric acid (0.6 M)

Three groups were established. Group A (n=12): guinea-pigs were exposed to phosphoramidon (10^{-3} M) for 10 min, followed by exposure to diprotin A (10^{-3} M) for 10 min and were then exposed to substance P (10^{-3} M) for 10 min with a 5 min monitoring period thereafter. Group B (n=5) was the control: guinea-pigs were exposed to vehicles for all drugs used in group A. Group C (n=11): guinea-pigs were exposed to citric acid for 10 min period and monitored for 5 min thereafter.

2.4.3. Challenge of guinea-pigs with citric acid (0.6 M) following pre-treatment with substance P (10^{-4} M) or bradykinin (10^{-4} M)

4 groups were established. In groups A (n=8) and B (n=8) guinea-pigs were exposed to substance P (10^{-4} M) and saline, respectively, and then immediately exposed to citric acid for 10 min followed by a 5 min monitoring period. In groups C (n=7) and D (n=7) guinea-pigs were exposed to bradykinin (10^{-4} M) and water, respectively, and then immediately exposed to citric acid for 10 min followed by a 5 min monitoring period.

2.5. Expression of results and statistical analysis

2.5.1. Cough

Values are given as means±S.E.M., and represent the total number of coughs during a 15 min period unless otherwise stated. The data were first analyzed for normal distribution. A non-parametric test was used when the data were found to be not normally distributed. The differences in the number of coughs between two treatment groups were analyzed using a non-parametric

test. The differences in the numbers of coughs between several treatment groups were analyzed using non-parametric one-way analysis of variance (ANOVA) followed by an ad hoc Dunn's test if significance was found. A P value <0.05 was taken as significant.

2.5.2. Bronchoconstriction

Airway obstruction was expressed as percent change in Penh. The data were first analyzed for normal distribution. The difference in the degree of airway obstruction, as measured by Penh, was determined by analyzing the whole time dependent % change in Penh using a non-parametric ANOVA followed by an ad hoc Dunn's test if significance was found.

A Sigmastat programme (SPSS, USA) was used to carry out all the statistical analyses.

3. Results

3.1. Effect of substance P on

3.1.1. Cough

Exposure of guinea-pigs to substance P at concentrations of 10^{-10} and 10^{-4} M did not cause a cough response in any of the animals in comparison with saline except that 3 out of the 11 animals had a cough response (2, 3 and 8 coughs respectively) following 10^{-3} M of substance P (Table 1).

3.1.2. Bronchoconstriction

Exposure of guinea-pigs to substance P at concentrations of 10^{-10} M or 10^{-4} M did not cause any bronchoconstriction when compared to saline exposed animals (Fig. 1). However, guinea-pigs challenged with substance P (10^{-3} M) demonstrated a significant degree of bronchoconstriction, compared to other groups, which developed gradually and peaked 3 min after the end of exposure (P<0.05; Fig. 1).

Table 1
The table shows the of effect substance P (10^{-10}, 10^{-4} and 10^{-3} M) on cough, the effect of phosphoramidon and diprotin A pre-treatment on substance P-induced cough

Treatment	Number of coughs
Substance P (10^{-10} M)	0
Substance P (10^{-4} M)	0
Substance P (10^{-3} M)	1.1±0.6
Vehicle (0.9% saline)	0
Substance P (10^{-3} M) post-phosphoramidon and diprotin	2.8±0.9
Citric acid (0.6 M)	10.9±1.8*
Substance P (10^{-4} M)	0 (over 10 min only)
Citric acid (0.6 M) post-substance P (10^{-4} M)	11.7±1.8
Vehicle (0.9% saline)	0 (over 10 min only)
Citric acid (0.6 M) post-vehicle	12.8±1.5
Bradykinin (10^{-4} M)	0 (over 10 min only)
Citric acid (0.6 M) post bradykinin	25.8±2.5#
Vehicle (water)	0 (over 10 min only)
Citric acid (0.6M) post-vehicle	9.2±1.9

The table also shows the effect of citric acid (0.6 M) alone and citric acid pre- and post-substance P (10^{-4} M) and bradykinin (10^{-4} M) on cough. Data are expressed as means±S.E.M., (n=5–12). Total number of cough counts is for a period of 15 min unless otherwise stated.

* P<0.05 vs. substance P challenge (10^{-10}–10^{-3} M) and substance P (10^{-3} M) post-phosphoramidon and diprotin A pre-treatment.

\# P<0.05 vs. bradykinin, water and citric acid post water exposures.

3.2. Effect of pre-treatment with phosphoramidon and diprotin A on substance P-induced

3.2.1. Cough

No cough was recorded in the vehicle group or during phosphoramidon or diprotin A challenges. Challenge of guinea-pigs, pretreated with phosphoramidon and diprotin A, with substance P resulted in 6 out of 12 guinea-pigs coughing with the overall mean cough for this group being 2.8±0.9. However, this was not significantly different when compared with either the vehicle challenged group or the substance P alone group and was significantly less than the citric acid-induced cough

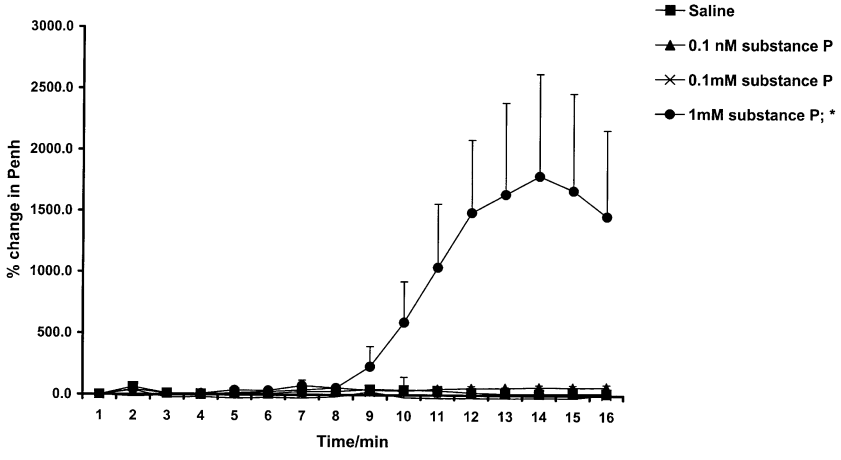

Fig. 1. Effect of increasing doses of inhaled substance P (10^{-10}, 10^{-4}, and 10^{-3} M) on airway caliber. Data are expressed as means±S.E.M., (n=5–11). *P<0.05 vs. saline, 10^{-10} and P 10^{-4} M challenged guinea-pigs.

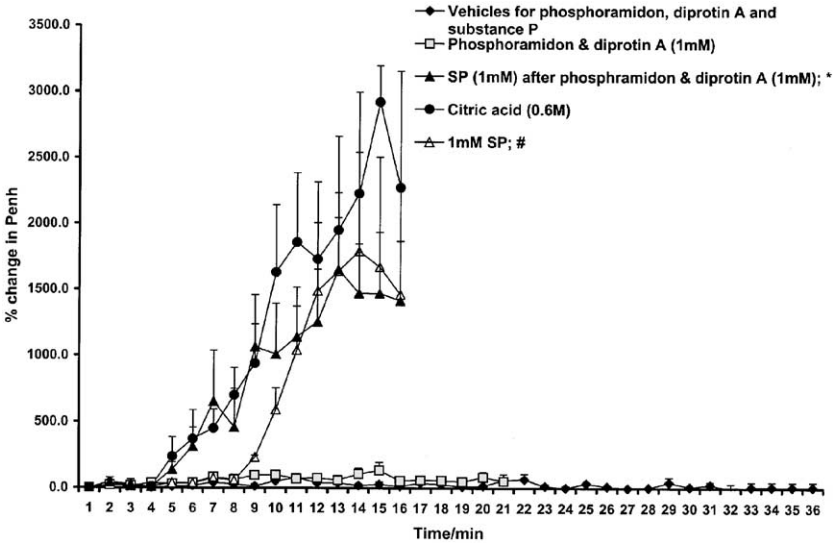

Fig. 2. Effect of pre-treatment with a combination of phosphoramidon and diprotin (10^{-3} M) on substance P-induced bronchoconstriction. Data are expressed as means±S.E.M., (n=5–12). The figure also shows citric acid-induced bronchoconstriction and the effect of phosphoramidon and diprotin exposures on airway caliber. The effect of substance P alone on airway caliber is shown for comparison. *P<0.05 vs. vehicle group and during phosphoramidon and diprotin A challenge. $^{\#}P$<0.05 vs. phosphoramidon and diprotin A pretreated and substance P challenged guinea-pigs and also vs. citric acid challenged guinea-pigs.

(2.8±0.9 vs. 10.9±1.8; P<0.05; Table 1). Indeed, citric acid-induced cough was significantly greater than cough induced in the substance P (10^{-10}–10^{-3} M) challenged guinea-pigs (P<0.05; Table 1).

3.2.2. Bronchoconstriction

Phosphoramidon and diprotin A did not induce bronchoconstriction per se (P>0.05; Fig. 2). However, challenge with substance P, following pre-treatment with phosphoramidon and diprotin A, resulted in a significant bronchoconstriction compared with vehicle challenged guinea-pigs (Fig. 2) with 2 animals having to be removed from the exposure chamber due to serious respiratory distress. The phosphoramidon and diportin A pre-treatment significantly potentiated the substance P-induced bronchoconstriction (P<0.05; Fig. 2). This was mainly evident in the early part of the substance P-induced bronchoconstriction. In fact, the phosphoramidon and diportin A enhanced bronchoconstriction was not significantly different from that induced by citric acid (P>0.05; Fig. 2). The ability substance P alone to induce bronchoconstriction is shown in Fig. 2 for comparison.

3.3. Effect of substance P (10^{-4} M) on citric acid-induced

3.3.1. Cough

Exposure of guinea-pigs to substance P for 10 min did not affect the citric acid-induced cough when compared to saline pre-treated guinea-pigs (11.7±1.8 vs. 12.8±1.5; P>0.05; Table 1).

3.3.2. Bronchoconstriction

Exposure of guinea-pigs to citric acid induced a significant degree of bronchoconstriction compared to saline or substance P exposure (P<0.05; Fig. 3). Moreover, pre-exposure of guinea-pigs to substance P resulted in a more significant degree of bronchoconstriction in response to citric acid when compared to saline pre-exposure (P<0.05; Fig. 3).

3.4. Effect of bradykinin (10^{-4} M) on citric acid induced

3.4.1. Cough

Exposure of guinea-pigs to bradykinin (10^{-4} M) did not cause any cough response (Table 1). However pre-exposure of guinea-pigs to bradykinin for 10 min significantly enhanced the citric acid-induced cough response compared to vehicle pre-exposed guinea-pigs (9.2±1.9 vs. 25.8±2.5; P<0.05; Table 1).

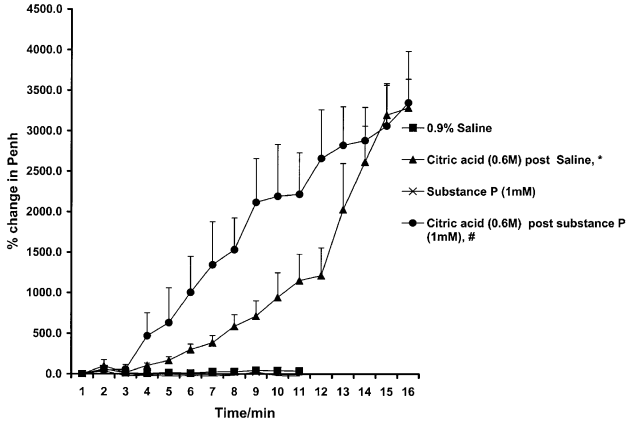

Fig. 3. Effect of substance P (10^{-4} M) on airway calibre and on citric acid (0.6 M)-induced airway bronchoconstriction. Data are expressed as means±S.E.M., (n=8). *P<0.05 vs. saline and substance P groups. $^{\#}P$<0.05 vs. citric acid post-saline exposure.

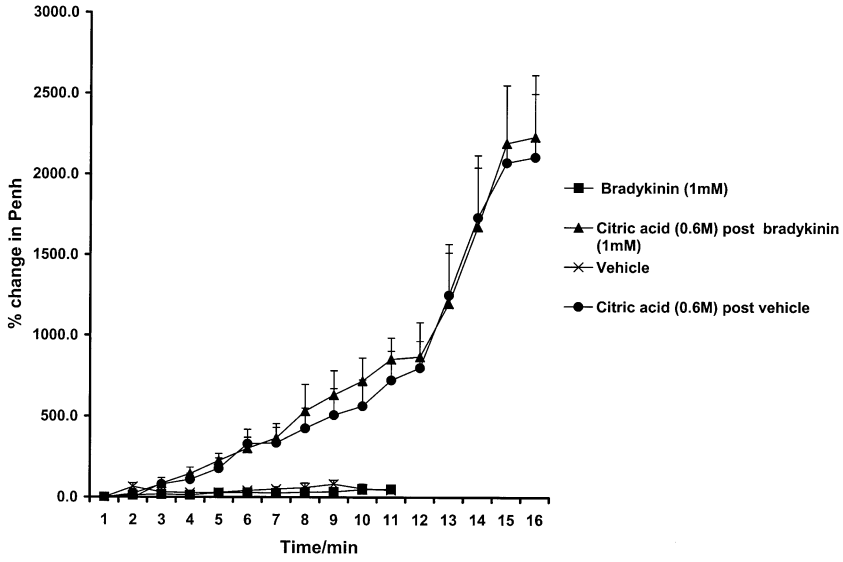

Fig. 4. Effect of bradykinin (10^{-4} M) on airway caliber and on citric acid (0.6 M)-induced bronchoconstriction. Data are expressed as means+S.E.M., (n=7).

3.4.2. Bronchoconstriction

Exposure of guinea-pigs to citric acid induced a significant degree of bronchoconstriction compared to vehicle or bradykinin exposure (P<0.05; Fig. 4). Bradykinin exposure neither induced bronchoconstriction compared to vehicle exposed guinea-pigs (P>0.05) nor significantly altered the citric acid-induced bronchoconstriction (P>0.05; Fig. 4).

4. Discussion

The results presented in this study show that substance P administration did not significantly affect the cough reflex at any of the doses used although, at the highest dose, substance P was found to induce a significant degree of bronchoconstriction. Pre-exposure of animals to a combination of phosphoramidon and diprotin A significantly enhanced the substance P-induced bronchoconstriction without affecting the cough. Pre-exposure of guinea-pigs to substance P had no effect on the citric acid-induced cough but significantly enhanced the degree of citric acid-induced bronchoconstriction. In contrast, pre-exposure of guinea-pigs to bradykinin significantly increased the cough response to citric acid but had no effect on the citric acid-induced bronchoconstriction. These data do not support a major peripheral role for substance P in the cough reflex but demonstrate that substance P can selectively sensitize afferent nerves to enhance their efferent function. In contrast bradykinin can selectively sensitize vagal afferent function.

The finding that neither the administration of substance P alone, in the dose range used, nor following pre-treatment with phosphoramidon and diprotin A, induced a consistent and significant cough response was somewhat surprising. In fact the cough response in these groups was significantly less than that evoked by citric acid. As substance P has been reported to stimulate RARs (Prabhakar et al., 1987; Matsumoto et al., 1994), albeit this appears to be an indirect effect (Bonham et al., 1996), its inability to induce cough, in this study, is not consistent with the suggestion that RARs are important in mediating cough.

These findings contradict previously published data where very small doses of substance P, as small as femto molar, were reported to potently induce a cough response (Kohrogi et al., 1988; Takahama et al., 1993, 1995). Our results are also at odds with studies showing that pre-treatment with phosphoramidon alone evokes cough (Takahama et al., 1995). The lack of effect of substance P on cough in our study was certainly not due to poor penetration of substance P into the lower airways as substance P(10^{-3} M)-induced bronchonchocontriction.

Our data would therefore suggest that substance P is not a potent peripheral tussive stimulus in agreement with other studies where no cough response was noted in response to aerosolized substance P (Joos et al., 1987; Fox et al., 1996a,b; Moreaux et al., 2000).

The reason for the discrepancies in the literature regarding the effect of aerosolized substance P on cough is not known. However, a possible explanation is differences in experimental conditions between laboratories. For example, in our study, and in others (Joos et al., 1987; Moreaux et al., 2000) where no cough was noted, substance P was dissolved in 0.9% saline whereas in studies reporting a cough response, the stock solution was made up in 0.1 N acetic acid (Kohrogi et al., 1988; Takahama et al., 1995). It is plausible that the acidic nature of the vehicle used in the latter studies may have been partly responsible for the observed cough. Furthermore, all the substance P used in our experiments was made fresh whereas in the other studies, it appears the stock solution was stored in the fridge

which may allow the development of contaminants (Kohrogi et al., 1988).

Although our data and that of others question the significance of the peripheral role of substance P in evoking cough, other data clearly support a role of substance P. Thus, the peptide FK888 and non-peptide neurokinin NK_1 receptor antagonists (*S*)-1-(2{3-{3,4-dichlorophenyl}-1-{3-isopropoxyphenylacetyl]piperidin-3yl]ethyl)-4-phenyl-1-azoniabicyclo (SR 140333) and (+),(2*R*,3*R*)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP99,994) have been shown to inhibit cough induced by stimuli such as smoke, citric acid, capsaicin or histamine (Sekizawa et al., 1995; Yasumitsu et al., 1996; Bolser et al., 1997; Moreaux et al., 2000). More recently, we have reported that the non-peptide neurokinin NK_1 receptor antagonist 1-benzoyl-2-benzyl-4-aminopiperidine (NKP608) and the dual neurokinin NK_1 and NK_2 receptor antagonist, ((1*R*,3*R*,2*E*)-N-[3, 4-dichlorobenzyl)]-4-[(hexahydro-2-oxo-1*H*-azepin-3-yl)amino]-*N*-methyl-3,5 bis(trifluoromethyl) benzamide) (DNK333), potently inhibit citric acid-induced cough in guinea-pigs (El-Hashim et al., 2001, 2004). So the question is how are these seemingly conflicting findings reconciled.

An alternative hypothesis, to an airway site of action for substance P, is that substance P is involved in mediation of cough through interaction with central NK_1 receptors and that the NK_1 receptor antagonists are blocking cough through interaction with these central receptors. Evidence for this is based on the fact that substance P is found in nerve terminals in the nucleus tractus solitarius (Davis and Smith, 1999) in the brain stem (the region postulated to be the cough centre), and the region of first synapse for airway vagal afferents (Helke et al., 1981). Furthermore, NK_1 receptors are richly expressed in the nucleus tractus solitarius (Davis and Smith, 1999). Moreover, central administration of CP99,994, by the intra cerebroventricular route (i.c.v.), in guinea-pigs resulted in a significant inhibition of cough response to capsaicin suggesting a central action (Bolser et al., 1997). It is therefore plausible that the limited cough response noted at the highest dose of substance P, with and without phosphoramidon and diprotin, may likely be due to activation of central NK_1 receptors.

Interestingly, we also found that the cough response to citric acid was unaffected by the pre-exposure to substance P. This finding contrasts with previous reports in pigs, where substance P was shown to enhance the cough reflex to citric acid (Moreaux et al., 2000) and to be involved in the enhancement of cough induced by angiotensin converting enzyme inhibitors (Moreaux et al., 2001). The reason for this discrepancy is not known but is certainly not due to a low level of substance P reaching the airways in our study. Nonetheless, this lack of enhancement of the cough reflex by substance P was not very surprising to us since C-fibres do not appear to fire action potentials in response to substance P (Fox et al., 1996a; Undem and Carr, 2001).

Our results also showed that bradykinin did not directly induce a cough response at the 10^{-4} M or even 10^{-3} M (data not shown). However, unlike substance P, bradykinin did significantly enhance the citric acid-induced cough. A role for bradykinin in cough enhancement has long been associated with hypertensive patients on angiotensin converting enzyme inhibitors therapy (Smith, 2002). The exact mechanism by which bradykinin induces sensitization of the cough reflex is not known. However, some studies have shown that bradykinin can activate RARs (Hargreaves et al., 1992, 1993) and that RARs are indeed involved in mediation of cough (Canning, 2002). Although we cannot completely rule out the involvement of RARs in the bradykinin enhanced cough response, the lack of cough response to bradykinin or indeed substance P would suggest that RARs may not be involved in the cough response in this model. An alternative explanation is that bradykinin may be sensitizing the C-fibres (Carr et al., 2003; Kollarik and Undem, 2004). Bradykinin has been shown to enhance capsaicin-induced action potentials, in vagal afferents, but not those induced by hypertonic saline (Fox et al., 1996b).

Our results also showed that substance P (10^{-3} M) induced significant airway bronchoconstriction, although the extent of this response varied. Moreover, pre-treatment with both phosphoramidon and diprotin A increased the airway bronchoconstriction induced by substance P which necessitated the removal of some guinea-pigs from the exposure chambers. This increase was mostly evident during the earlier part of the nebulization of substance P. A similar degree of variability in airway response and level of distress, to substance P, has been observed previously (Koch et al., 1999).

Our data confirm previous in vivo studies, both in animal and human, showing bronchoconstrictor effects of substance P (Andersson et al., 1982; Lundberg et al., 1983; Joos et al., 1987, 2000; Shore et al., 1988; Crimi et al., 1990; Corboz et al., 2003). However, it is interesting to note that the effect of neurokinin A, via activation of NK_2 receptors, on airway caliber appears to be greater than the effects of substance P, acting via NK_1 receptors, in both guinea-pigs and human airways (Joos et al., 1987, 2000; Corboz et al., 2003; El-Hashim et al., 2004). Hence, as substance P is not very selective for NK_1 receptors, it is possible that the bronchoconstriction induced by substance P is mediated partly via NK_2 receptors.

Our data also show that substance P (10^{-4} M) pre-treatment significantly enhanced the citric acid-induced airway obstruction. This increase in airway obstruction to citric acid, following substance P pre-treatment, is unlikely to be due to substance P-induced airway bronchospasm or overt airway inflammation as our data do not show any significant changes in the airway caliber, to 10^{-4} M of substance P, that would account for this. Although the increase in airway response following substance P is temporally different to that which has been reported previously in the literature to numerous bronchoconstrictor agents (Cheung et al., 1994; Boichot et al., 1996; Daoui et al., 1997), the underlying mechanism may be similar.

Moreover, it is also possible that the substance P pre-exposure may be sensitizing the efferent function of airway nerves to citric acid. Indeed, several mechanisms are possible, such as facilitation of acetylcholine release from cholinergic nerves (Colasurdo et al., 1995) and enhancement of mast cell degranulation (Baumgarten et al., 1996; Hua et al., 1996). In addition, the enhanced airway obstruction could also be mediated centrally (Mazzone and Canning, 2002).

Our data also showed that, in contrast to substance P, bradykinin neither induced airway bronchoconstriction in the guinea-pigs nor enhanced the citric acid-induced airway bronchoconstriction. Similarly, in non-asthmatic humans, bradykinin has minimal airway effects when administered by the inhaled route (Barnes, 1992; Trifilieff et al., 1993).

In summary, our data clearly show that the role of substance P in cough is most probably not through an interaction with peripheral neurokinin receptors. Finally, although neither bradykinin nor substance P appears to be directly involved in evoking a cough response, bradykinin is capable of enhancing the cough reflex. Furthermore, our data show that, in healthy guinea-pigs, substance P, but not bradykinin, plays an important role in enhancing the airway response to bronchoconstricting agents. These data therefore show that inflammatory mediators can differentially sensitize the airways to tussive and bronchoconstrictor stimuli.

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